Environmental Health Perspectives
Vol. 22, pp. 141–144, 1978

Genetic Variability and Extrapolation from Animals to Man: Some Perspectives on Susceptibility to Chemical Carcinogenesis from Aromatic Amines

by Wendell W. Weber*

I would like to consider the problem of chemical carcinogenesis arising from exposure to certain aromatic amines as a basis for my remarks about the predictive value for man of data obtained in other species and biological systems. This example was chosen because it illustrates the complexity of extrapolation very well, and there is new knowledge derived from a variety of disciplines including biochemistry, molecular biology, microbial and human genetics and pharmacology and from various levels of biological organization which must be brought together to enable us to predict the risks to man from exposure to these environmental agents.

Aromatic amines are one of the principal groups of chemical carcinogens. They were first discovered to be carcinogenic in man as a result of industrial exposure followed by a 15–20 year latent period. Aromatic amines which produce tumors in animals are not in themselves carcinogenic, but they become so after they have been converted *in vivo* to more reactive substances. Metabolism of carcinogenic arylamines is complex and may include ring hydroxylation, *N*-hydroxylation, conjugation of the ring and *N*-hydroxyl metabolites, and *N*-acylation of the amine function. Deacylation of *N*-acylated arylamines is also observed. The major route for metabolism of carcinogenic arylamines in many species involves *N*-acetylation of the amine group (*1*).

*Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan 48109.

The information that I would like to discuss can be divided into three parts: (1) that which tells us about the chemical nature of mutagenic and carcinogenic metabolites of these amines; (2) that which has enabled us to test and classify these substances according to their mutagenic properties and carcinogenic potential; and (3) that which has pinpointed specific individual differences which can affect metabolic capacity of exposed persons to generate the ultimate carcinogen(s) and hence alter their susceptibility to mutagenic and carcinogenic changes.

Aminofluorene (AF) (Fig. 1) is a well known representative aromatic amine which causes tumors in animals (*2*). It is also now well established that *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF) is an *in vivo* metabolite of AF (*3*), but even this compound is probably not the ultimate carcinogen. *N*-Hydroxylation of AAF is catalyzed by a microsomal oxidative enzyme which involves the heme-protein catalyst, P_1-450, or as it is now called, P-448. Reactions of this sort are not unusual, since most drugs and foreign substances are extensively metabolized by the body before they are eliminated by oxidation, reduction, or hydrolysis, or by conjugation to form acetyl, glucuronide, or sulfate derivatives. In fact, many foreign substances are metabolized by more than one of these reactions either simultaneously or consecutively. Generally speaking, these transformations are catalyzed by special enzymes in the liver and other tissues of the body, and they are subject to variation on a hereditary basis. Because of the marked variation that oc-

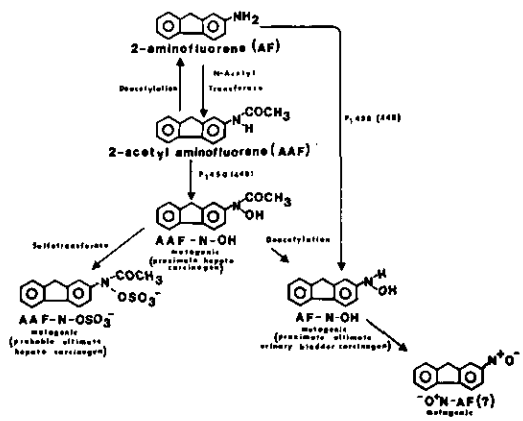

FIGURE 1. Metabolic activation of dangerous amines.

curs between different animals and man, no one species can serve as a model for the metabolism for any foreign compound in all humans, nor does any single species exactly mimic the metabolism of all drugs in any individual (*4*). It must also be kept in mind that the levels of activities of these drug metabolizing enzymes are generally inducible by phenobarbital and with specificity by some hydrocarbons (e.g., 3-methylcholanthrene), and they are modulated by a variety of other environmental factors including drugs and diet. The extent of enzyme induction may also be genetically regulated (*5*).

Within the past few years the patterns of metabolism of numerous hydrophobic substrates including various carcinogenic and toxic exogenous chemicals have been examined after exposure to different enzyme inducers in an experimental mouse genetics model. Variations in the patterns of metabolism have been identified with variation in the genetic expression of cytochrome P-448 formation (*6*). In early work with this mouse genetics model, Nebert and his colleagues showed that the induction of aryl hydrocarbon hydroxylase activity and the formation of new cytochrome P-448 both display Mendelian inheritance as an autosomal dominant and are genetically coregulated in that species. Recently, they have extended these findings in this system to several known carcinogens, including AAF (*7*). These studies are especially pertinent here, because the genetic differences in capacity to metabolize foreign compounds were combined with the bacterial mutagenesis assay developed by Ames (*8*) to evaluate the metabolic activation of AAF to a frame-shift mutagen. The metabolic activation process of AAF was found to be inherited in Mendelian fashion as an autosomal dominant and involved the same genes as those responsible for aryl hydrocarbon hydroxylase activity and the formation of new cytochrome P-448 in the mouse. Thus the mutagenic effects of AAF appear to be associated with genetically mediated increases in microsomal oxidative enzyme activity associated with a specific cytochrome, P-448.

As data continues to accumulate, it is becoming clearer that alternate metabolic products may be formed from the same substrate under the stimulus of different inducers, presumably because of catalytic differences in variant forms of the cytochromes induced. In the future, presumably it will be possible, by use of this experimental genetics model system in combination with inhibitors which can block alternative inducible pathways selectively, to evaluate the relative importance of individual pathways in augmenting or decreasing mutagenesis associated with specific agents such as AAF.

Use of the bacterial mutagenesis assay to assess the metabolic activation of AAF and other hazardous substances (Table 1) is somewhat controversial, because it involves a kind of double-barreled extrapolation. One is the extrapolation from mutant tester bacterial (*Salmonella typhimurium*) strains to eukaryotic mammalian cells. The other is the extrapolation from mutagenesis to carcinogenesis. Evidence is accumulating, much of it obtained with this test, that there is a high correlation between carcinogenicity and mutagenicity: 90% (156/174) of carcinogens tested were found to be mutagenic. In addition, 46 common biochemicals that are noncarcinogens, or presumed noncarcinogens, have been tested and found negative (*9*). On the other hand, many carcinogens have been suggested to be nonmutagenic, making it seem that there was no necessary relationship between carcinogenicity and mutagenicity (*10*). This apparent discrepancy has been reduced by the discovery of metabolic activation *in vivo*, which has provided strong support for somatic mutagenesis as an attractive hypothesis to

Table 1. Assay of amines by the Ames Salmonella/microsome test system.[a]

Amine	Mutagenicity	Carcinogenicity
2-Aminofluorene	++	+
6-Aminochrysene	+	+
Proflavin	+	?
2-Aminoanthracene	++	+
1-Aminoanthracene	+	+
α-Naphthylamine	+	0
β-Naphthylamine	+	+
4-Aminobiphenyl	+	+
2-Aminobiphenyl	+	?
Benzidine	+	+
Aniline	0	?

[a]Data of McCann (*9*).

explain chemical carcinogenesis. Any program of cancer prevention from chemical carcinogens requires simple, rapid and inexpensive screening methods as complements to expensive, long-term animal tests to identify dangerous chemicals and the Salmonella/microsome test is an elegant approach which satisfies these requirements. The utility and limitations of this test and other tests which are similar in principle but use tester strains of mammalian cells (*11*) for detecting chemicals likely to be environmental mutagens or carcinogens for humans is continuing.

Inspection of the pathway for metabolic activation of AF in Figure 1 indicates several other potential sites for genetic variation. Clearly, the capacity for deacetylation is enzymatically mediated and subject to genetic variability which could alter the rate of formation of an ultimate carcinogen. A similar comment can be made about the sulfation of AAF which is mediated by a sulfotransferase. Very little appears to have been reported about the range of variability of either of these activating reactions.

The initial step in the activation of AF is acetylation of the aromatic amine group. A genetic polymorphism in the acetylation of drugs which contain either an aromatic amine or a hydrazine group has been known since its recognition by Hughes more than twenty years ago in studies of patients experiencing chronic toxicity from isoniazid (*12*). Genetic studies in human families (*13, 14*) showed that persons could be classified into two major genetic subgroups, termed rapid and slow isoniazid acetylators (Fig. 2). Acetylation of isoniazid is controlled by two autosomal allelic genes, and slow acetylators are homozygous for the slow recessive gene. Differences in the rate of acetylation of isoniazid are attributable to different amounts of drug acetylating enzyme (*N*-acetyltransferase) in liver. A genetic polymorphism of a similar sort also exists in rabbits and the rate of elimination of isoniazid by acetylation is highly correlated in both species with the amount of isoniazid *N*-acetyltransferase in liver.

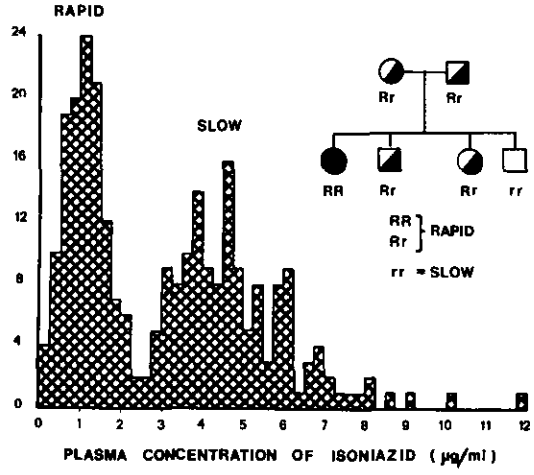

FIGURE 2. Bimodal distribution of plasma isoniazid concentrations (*14*).

There are several drugs in wide medical use today which must undergo acetylation prior to their elimination. The rate of acetylation of some of these drugs is controlled mainly by the same genes as that for isoniazid while the rate of acetylation of several others is not (Table 2). Our laboratory has been interested in obtaining information about the basis of this genetic trait and its consequences for man for several years (*15*). Thus, it was of interest to us to ask whether the rate of acetylation of any of the mutagenic and carcinogenic amines might be subject to variation in the same way as isoniazid and we devised a simple screening test to test this hypothesis. The test involves the determination of the rate of acetylation of the hazardous amine with liver homogenates prepared from rapid and slow acetylator rabbits, and comparison with the rates of acetylation of our standard "polymorphic" substrate, sulfamethazine, and our standard "monomorphic" substrate, *p*-aminobenzoic acid. Our findings show that aminofluorene resembles sulfamethazine, and thus behaves as a "polymorphic" substrate like isoniazid in this system (Table 3). Other amines selected from the list of mutagenic and carcinogenic amines (*9*) which we have tested in this system are benzidine, α-naphthylamine, and β-naphthylamine, and they also are "polymorphic" substrates (*16*).

Table 2. Drugs inactivated by acetylation in man and rabbit.

I. Drug substrates for polymorphic (isoniazid) liver *N*-acetyltransferase
Isoniazid
Sulfamethazine
Hydralazine
Diaminodiphenylsulfone
Sulfapyridine
Procainamide
II. Drugs acetylated mainly by other *N*-acetyltransferases
p-Aminobenzoic acid (PABA)
p-Aminosalicylic acid
Sulfanilamide

Thus, it appears that individuals that are genetically rapid acetylators of isoniazid might also be expected to transform AF and other hazardous amines to activated carcinogenic forms more

Table 3. Polymorphic acetylation of dangerous amines with rabbit liver *N*-acetyl transferase.[a]

Amine substrate	K_m, M	$\bar{R}$ Rate/$\bar{S}$ Rate [b]
Sulfamethazine	4.4×10^{-4}	287 : 1
p-Aminobenzoic acid	6.9×10^{-5}	7 : 1
α-Naphthylamine	8.2×10^{-5}	539 : 1
β-Naphthylamine	4.1×10^{-5}	312 : 1
2-Aminofluorene	8.0×10^{-6}	579 : 1
Benzidine	6.0×10^{-6}	555 : 1

[a][AcCoA] = 5×10^{-4} M.
[b]$\bar{R}$ = rapid INH acetylator; $\bar{S}$ = slow INH acetylator.

rapidly than slow acetylators. These observations should be confirmed with enzyme preparations from rapid and slow human liver before they can be taken as conclusive, and we are currently continuing our assessment of the possible role of this genetic polymorphism as a human determinant of susceptibility to chemical carcinogenesis from dangerous aromatic amines.*

*Subsequent investigations with homogenates of human liver *N*-acetyltransferase obtained from rapid and slow acetylators have confirmed these findings for man. These results will be the subject of a more detailed report to be published elsewhere.

REFERENCES

1. Williams, R. T. The metabolism of aromatic amines. In: Detoxication Mechanisms, 2nd ed., Wiley, New York, 1959, p. 428.
2. Miller, J. A., and Miller, E. C. The metabolic activation of carcinogenic aromatic amines and amides. Progr. Exptl. Tumor Res. 11: 273 (1969).
3. Miller, E. C., Miller, J. A., and Enomoto, M. The comparative carcinogenicities of 2-acetylaminofluorene and its *N*-hydroxy metabolite in mice, hamsters, and guinea pigs. Cancer Res. 24: 2018 (1964).
4. Gillette, J. R. Comparative metabolism and the choice of experimental animals. In: 5th ICLA Symposium, Gustav Fischer Verlag, Stuttgart, 1973, p. 1.
5. Kellermann, G., Luyten-Kellermann, M., and Shaw, C. R. Genetic variation of aryl hydrocarbon hydroxylase in human lymphocytes. Am. J. Human Genet. 25: 327 (1973).
6. Nebert, D. W., et al. Genetic expression of aryl hydrocarbon hydroxylase activity in the mouse. J. Cell. Physiol. 85: 393 (1975).
7. Felton, J. S., and Nebert, D. W. Mutagenesis of certain activated carcinogens *in vitro* associated with genetically mediated increases in monooxygenase activity and cytochrome P_1450. J. Biol. Chem. 250: 6769 (1975).
8. Ames, B. N., et al. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. (U.S.) 70: 2281 (1973).
9. McCann, J., et al. Detection of carcinogens as mutagens in the salmonella microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. (U.S.) 72: 5135 (1975).
10. Goldstein, A., Aronow, L., and Kalman, S. M. Principles of Drug Action: The Basis of Pharmacology, 2nd ed., Wiley, New York, 1974, p. 682.
11. Huberman, E., et al. Identification of mutagenic metabolites of benzo[a]pyrene in mammalian cells. Proc. Nat. Acad. Sci. (U. S.) 73: 607 (1976).
12. Hughes, H. B., et al. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. Amer. Rev. Tuberc. 70: 266 (1954).
13. Knight, R. A., Selin, M. J., and Harris, H. W., Genetic factors influencing isoniazid blood levels in humans. Trans. Conf. Chemother. Tuberc. 18: 52 (1959).
14. Evans, D. A. P., Manley, K. A., and McKusick, V. A. Genetic control of isoniazid metabolism in man. Brit. Med. J. 2: 485 (1960).
15. Weber, W. W. Acetylation of drugs. In: Metabolic Conjugation and Metabolic Hydrolysis. W. H. Fishman, Ed., Academic Press, New York, 1973, p. 249.
16. Glowinski, I. B., Radtke, H. R., and Weber, W. W. Genetic susceptibility to chemical carcinogenesis from aromatic amines. Pharmacologist 18: 231 (1976).